Claims 1, 7, 9, 1 0, 12, 25, and 26 have been amended. Support for the amendments is found in the existing claims and the specification. Accordingly, the amendments do not constitute the addition of new matter. I respectfully request the entry of the amendments and reconsideration of the application in view of the amendments and the following remarks. I believe that entry of the present amendment will, at least, reduce the number of issues for appeal.

The specific changes to the specification and the amended claims are shown on a separate set of pages attaches hereto and entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment. On this set of pages, insertions are underlined and deletions are struck through.

Rejections under 35 U.S.C. §l12

My amendment specifies the temperature range in which the invention is to operate. The new temperature frame is both within the range that I set forth earlier as well as still allowing the invention to be viable. The most important aspect of this specification is that it is far above the -196°C required but other inventions of a related nature. The utility of this application lies in the invention's ability to vitrify and preserve specimens at temperatures previously not possible.

The Examiner further asserts that the term "cooling" is unclear, as it is a relative term with no reference point. However, it is clear from the specification that the "cooling" occurs from ambient temperatures to temperatures that are obtainable by use of a refrigerator. Additionally, since the applicant has specified the lower end of the temperature frame the term cooling must be in reference of drawing heat from a body that was previously at a higher temperature, most likely at an ambient temperature. In view of these arguments, it is respectfully requested that the Examiner reconsiders and withdraws the grounds of rejection for claim 1.

New matter

Claims 1, 4-10, 12-17, 25, and 26 are rejected under 35 112, first paragraph as containing new matter. I respectfully request that the Examiner look to the newly amended Claim 1, in which a vitrification step is specified. This mention does not introduce new material as it was always inherent and does not change the intention nor the process in the application but simply clarifies the step so that those educated in the relevant art would understand the meaning.

Rejections under 35 U.S.C. 4102

Claims 1, 4-7, 16, and 25 were rejected under §102(b) as being anticipated by Titterington et al. This reference teaches immersion of specimens in liquid nitrogen (-196' C) in the presence of sucrose (a non-permeating co-solute), glycerol (a permeating cryoprotectant) and Percoll (a non-permeating cryoprotectant). However, the disclosure of Titterington et al. is limited to rapid freezing to -196' C. Titterington et al. does not teach dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant, and then vitrifying the dehydrated specimen by cooling to a refrigerated or higher storage temperature, as recited in present Claim 1. My specification of temperature creates a definite distinction between Titterington et al. teaching exposing specimens to temperatures of -196°C and the present application teaching vitrification at above -80°C. Accordingly, I respectfully request withdrawal of the §102 rejection of Claims 1, 4-7, 16, and 25 based on Titterington et al.

Claims 1-6, 9, 10, 16, 18-24 and 25 remain rejected under 35 U.S.C. § 102 as being anticipated by Rall et al. Claims 18-24 have been canceled. Rall et al. discloses progressive or step-wise cooling of embryos down to -196' C (liquid nitrogen). My claims are drawn to vitrification at refrigeration temperatures, specified at above -80°C. Rall et al. cannot anticipate my claims as the disclosure of Rall et al. is drawn to the use- of much lower temperatures. In view of these arguments, withdrawal of this ground of rejection is respectfully requested.

Claims 1, 4-7, 9, 12-16, 25 and 26 remain/are rejected under 35 U.S.C. § 102 (b) as anticipated by U.S. Patent No. 5,364,756. The '756 patent teaches the use of very low temperatures, e.g., -196'C (see col. 17 lines 15-39 and Example 4 which teaches temperatures of -160'C. Again, the claimed method is not drawn to the use of such low temperatures. My method is directed to the use of temperatures, which can be achieved with the use of a refrigerator namely those temperatures are over -80°C. Since the specified temperature is above the vitrification or glass phase transition temperature, patent '756 cannot anticipate application. Withdrawal of this ground of rejection is respectfully requested.

Claims 1, 4-7, 9, 10, 12-17, 25, and 26 remain rejected under §102(e) as anticipated by U.S. Patent No. 5,800,978 to Goodrich. With the utmost respect, I disagree with the response of examiner to our argument, because of table 1 and example 1 and other examples in Goodrich et al (5/800,978) (please see copy of the patent enclosed). My invention discloses a method of vitrification, which is solidification without formation of ice crystals. In these examples, Goodrich is referring to frozen solutions, i.e. ice crystal have formed. What they list is Tg' (read Tg Prime) in example 1 and table 1 is the glass transition temperature of a maximum freeze dehydrated solution remaining during freezing between ices crystals in frozen specimens. Tg' is an important characteristic of frozen solution which everybody, including Goodrich used to determine the optimum condition of freeze-drying from a frozen state to which this invention is devoted. Therefore this reference does not anticipate my invention, which suggests vitrification with no formation of ice crystals. Consequently, Goodrich does not teach all of the elements of my claimed invention and the rejection under 35 U.S.C. § 102 may be properly withdrawn.

Rejections under 35 U.S.C. 4103

Claims 1, 4-10, 12-16, 25-26 are/remain rejected under 35 U.S.C. § 103(a) as unpatentable over U.S. 5,364,756 in view of 5,217,860 taken with U.S. 4,865,871 or Rall et al. and U.S. 5,879,876. The primary reference fails for the reasons given above in the response to the rejection under 35 U.S.C. § 102. The '756 patent teaches the use of very low temperatures, e.g., -196'C (see col. 17 lines 15-39 and Example 4 which teaches temperatures of -160°C). Again, my claimed method is not drawn to the use of such low temperatures. My method is directed to the use of temperatures above -80°C. None of the cited references alone or in combination teach or suggest vitrifying a dehydrated specimen by cooling to a refrigeration or higher storage temperature as recited in the amended claim 1. The '860 disclosure teaches temperatures of -135°C (col. 24, line 37). The '871 disclosure teaches the use of temperatures of -196°C (col. 9, lines 40-42). The '876 disclosure teaches "ultra low temperature" (col. 15, lines 7). None of the cited references teach or suggest vitrification at higher temperatures such as above -80°C. Since all claims depend from claim 1, which is neither taught nor suggested by the cited references as discussed above, the invention defined in claims 4-10, 12-16, 25 and 26 is also patentably distinguished

from the references, alone or in combination. I respectfully request the withdrawal of the rejection.

CONCLUSIONS

In view of the foregoing amendments and remarks, the present application is submitted as in condition for allowance, and such action is earnestly solicited. If any matters should remain, the Examiner is invited to contact the undersigned at the telephone number provided below.

Respectfully submitted,

Dated: Oct. 6 04

By:

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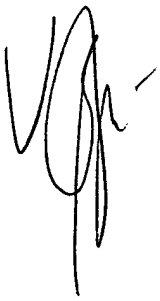

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Three times Amended) A method for cryopreserving a cell or tissue specimen without freezing (ice formation) comprising: (a) Dehydrating and loading the specimen by equilibrating ~~and thereby dehydrating the specimen~~ [with] in concentrated solutions comprising a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant, wherein the non-permeating co-solute effectively decreases the chemical potential of the permeating cryoprotectant thereby thermodynamically limiting the maximum amount of the permeating cryoprotectant ~~which permeates into the~~ loaded inside the cells of the specimen; ~~and vitrifying the dehydrated specimen, by cooling to a refrigeration or higher storage temperature~~. and (b) vitrifying (transforming to the solid amorphous state) the dehydrated specimen by cooling to a refrigerating storage temperature above –80°C; and (c)rehydrating and unloading the specimen after storage at the refrigerating temperature and subsequent warming to 0°C or above.

7. (Three times Amended) The method of claim 1, wherein the total concentration of non-permeating co-solute in any preserved specimen is between ~~0.1~~ 0.3 and 0.7 mol/l.

9. (Three times Amended) The method of claim 1, wherein-dehydrating and loading the specimen is performed in two or more stages of contacting the specimen with increasingly higher concentrations of the permeating cryoprotectant and the co-solute.

10. (Three times Amended) The method of claim 1, wherein dehydrating and loading the specimen is performed by simultaneously increasing concentrations of both permeating cryoprotectant and the co-solute from an initial concentration to a final concentration according to a desired profile.

12. (Three times Amended) The method of claim 1, further comprising rehydrating and unloading the specimen by ~~contacting the vitrified specimen with~~ equilibration in [with] a rehydration solution comprising a non-permeating rehydration co-solute which effectively decreases the chemical potential of a permeating rehydration cryoprotectant.

25. (Twice Amended) The method of claim 1, wherein the non-permeating co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, amino acid, and a disaccharide.

26. (Twice Amended) The method of claim 12, wherein the non-permeating rehydration co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, amino acid, and a disaccharide.